ELSEVIER

European Journal of Pharmacology 419 (2001) 93–97

ejp

www.elsevier.nl/locate/ejphar

Short communication

TY-12533, a novel Na^+/H^+ exchange inhibitor, prevents myocardial stunning in dogs

Kazuyuki Aihara [a,b], Hiroaki Hisa [a,*], Jun Sasamori [b], Fumiya Yoneyama [b], Fuminari Yamaguchi [b], Isamu Satoh [b], Susumu Satoh [a]

[a] *Laboratory of Pharmacology, Graduate School of Pharmaceutical Sciences, Tohoku University, Aobayama, Sendai 980-8578, Japan*
[b] *Drug Research Department, Fukushima Research Laboratories, Toa Eiyo Ltd., Iizaka, Fukushima 960-0280, Japan*

Received 14 March 2001; accepted 30 March 2001

Abstract

Anesthetized open-chest dogs were subjected to 15-min myocardial ischemia followed by 2-h reperfusion to induce myocardial stunning. A novel Na^+/H^+ exchange inhibitor 6,7,8,9-tetrahydro-2-methyl-5*H*-cyclohepta[*b*]pyridine-3-carbonylguanidine maleate (TY-12533), administered 10 min before or 10 min after start of ischemia (3 mg/kg/10 min, i.v.), did not affect reductions in regional myocardial wall thickening, blood flow and pH during ischemia, but it significantly improved recovery of the wall thickening and blood flow after reperfusion. These results indicate that TY-12533, even when administered during ischemia, could prevent myocardial stunning without affecting myocardial dysfunction or acidosis induced by brief ischemia.

Keywords: TY-12533; Na^+/H^+ exchange inhibitor; Myocardial wall thickening; Myocardial blood flow; Myocardial pH

1. Introduction

Brief period of myocardial ischemia followed by reperfusion induces reversible myocardial dysfunction defined as myocardial stunning (Braunwald and Kloner, 1982). One of the major pathogenesis responsible for the stunning is intracellular Ca^{2+} overload (Bolli and Marbán, 1999). The Ca^{2+} overload after reperfusion is mediated via reverse-mode Na^+/Ca^{2+} exchange that is activated by intracellular Na^+ overload via Na^+/H^+ exchanger (Tani and Neely, 1989). Smart et al. (1995) reported that amiloride, a partial Na^+/H^+ exchange inhibitor, prevented the myocardial stunning induced by brief ischemia followed by reperfusion in dogs, but they concluded that the prevention by amiloride might not be due to inhibition of Na^+/H^+ exchange. There has been no clear notion whether inhibition of Na^+/H^+ exchange could suppress the myocardial stunning in vivo.

We have recently found a novel Na^+/H^+ exchange inhibitor 6,7,8,9-tetrahydro-2-methyl-5*H*-cyclohepta[*b*]pyridine-3-carbonylguanidine maleate (TY-12533) that is effective against myocardial ischemia/reperfusion-induced arrhythmia and infarction in rats (Aihara et al., 2000). In the present study, we investigated whether TY-12533 prevents ischemia/reperfusion-induced myocardial stunning in dogs by measuring regional wall thickening as an index of myocardial function. Furthermore, we simultaneously measured regional myocardial blood flow and pH in the ischemic area to evaluate the effect of TY-12533 in more detail.

2. Materials and methods

All animal experiments were reviewed and approved by the Experimental Animal Committee of Drug Research Department, Toa Eiyo (Fukushima, Japan).

2.1. General preparation

Harlan Sprague–Dawley/Ridgilan Beagle dogs (Ridgilan Research Farms, Madison, WI, USA) of either sex, weighing 6.9–13.2 kg, were anesthetized with pentobarbital sodium (30 mg/kg, i.v.). Anesthesia was maintained by i.v. infusion of pentobarbital sodium (3–5 mg/kg/h) throughout the experiments. The trachea was intubated and the animal was artificially ventilated with room air by a

* Corresponding author. Tel.: +81-22-217-6837; fax: +81-22-217-6835.
E-mail address: hhisa@mail.pharm.tohoku.ac.jp (H. Hisa).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)00952-9

respirator (SN-480-3, Shinano, Tokyo, Japan). Following cardiac parameters were measured using a polygraph system (polygraph 363, San-ei, Tokyo). The blood pressure and heart rate were measured via a polyethylene catheter inserted into the right femoral artery. To measure left ventricular end diastolic pressure (LVEDP) and the first derivative of left ventricle pressure (LV dP/dt), an angiographic catheter (4F, Cordis Europe, Roden, The Netherlands) was inserted into the left ventricle through the right femoral artery. The right femoral vein was cannulated for drug administration. Electrocardiogram was measured from standard limb lead II.

Left thoracotomy was performed between the fifth and sixth ribs, and the heart was suspended in a pericardial cradle. Volume and electrolytes balance of body fluid were maintained by i.v. infusion (0.5 ml/min) of electrolyte replenisher (Solita® T No. 3, Takeda Chemical Industries, Osaka, Japan). The left anterior descending coronary artery was dissected free from the surrounding tissue at the portion just distal to the first diagonal branch, and a nylon ligature was loosely snared around the dissected vessel. The myocardial ischemia was achieved by tightening the snare, and reperfusion was performed by releasing the snare.

2.2. Measurement of regional myocardial wall thickening

Regional myocardial wall thickening was measured by the method described by Ogawa et al. (1996). A single Doppler probe (DMT104S, Crystal Biotech, Holliston, MA, USA) was secured to the epicardium of the territory perfused by the left anterior descending coronary artery, and the probe was connected to a wall tracking module (WT-10, Crystal Biotech). The wall thickening was calculated as (end systolic thickness − end diastolic thickness)/end diastolic thickness. The wall thickening was measured for 10 beats and then averaged. The values are expressed as percentage from the value before ischemia.

2.3. Measurement of regional myocardial blood flow and tissue pH

Regional myocardial blood flow was measured using the hydrogen clearance technique (Van Wylen et al., 1990). A platinum wire (UHE-201C, Unique Medical, Tokyo) was embedded into the endocardium (about 8-mm depth) of the territory perfused by the left anterior descending coronary artery. Hydrogen (final concentration being 5%) was added to the room air ventilation mixture for 1 min. Regional hydrogen washout from the tissue was measured using a polarography (MHG-DI, Unique Medical). Regional myocardial blood flow was calculated by dividing ln(90/40) by the time required for the hydrogen level to fall from 90% maximum to 40% (data were obtained as ml/min/g), and the values are expressed as percentage from the value before ischemia.

Regional myocardial pH was measured by the method described by Ichihara et al. (1979). A micro glass electrode (IC-HRT, Inter Medical, Nagoya, Japan) was also embedded into the endocardium (about 8-mm depth) of the territory perfused by the left anterior descending coronary artery and a reference electrode was attached to the tho-

Table 1
Hemodynamics before and after ischemia in control and TY-12533 treated dogs

	Before ischemia	During ischemia	Time after reperfusion (min)			
			5	30	60	120
Mean blood pressure (mm Hg)						
Control	97 ± 6	94 ± 5	94 ± 6	96 ± 4	96 ± 4	101 ± 5
TY-12533-Pre	118 ± 4	117 ± 4	115 ± 5	113 ± 5	103 ± 9	101 ± 10
TY-12533-Post	107 ± 8	111 ± 7	112 ± 5	113 ± 5	109 ± 5	103 ± 7
Heart rate (beats / min)						
Control	141 ± 3	150 ± 8	139 ± 4	142 ± 5	138 ± 6	133 ± 6
TY-12533-Pre	150 ± 6	147 ± 6	143 ± 6	149 ± 7	143 ± 8	146 ± 8
TY-12533-Post	159 ± 4	160 ± 5	158 ± 6	157 ± 6	155 ± 5	155 ± 3
Left ventricular end diastolic pressure (mm Hg)						
Control	6.2 ± 0.7	9.4 ± 1.1[a]	8.6 ± 1.1[a]	5.8 ± 0.5	5.8 ± 0.8	6.6 ± 0.8
TY-12533-Pre	6.0 ± 0.8	9.0 ± 1.4[a]	7.4 ± 1.4	6.0 ± 0.5	5.0 ± 0.7	5.0 ± 0.5
TY-12533-Post	5.4 ± 1.4	8.8 ± 1.0[a]	7.0 ± 0.6	6.2 ± 1.2	5.0 ± 1.1	4.6 ± 0.7
Left ventricular dP / dt ($\times 10^3$ mm Hg / s)						
Control	2.8 ± 0.1	2.4 ± 0.1[a]	2.4 ± 0.1[a]	2.5 ± 0.1[a]	2.4 ± 0.1[a]	2.2 ± 0.1[a]
TY-12533-Pre	3.2 ± 0.3	3.0 ± 0.1	2.9 ± 0.1	2.7 ± 0.2	2.8 ± 0.1	2.5 ± 0.1[a]
TY-12533-Post	3.2 ± 0.3	2.9 ± 0.3	2.9 ± 0.3	3.0 ± 0.3	3.0 ± 0.3	2.8 ± 0.2

Each value represents the mean ± S.E.M. TY-12533 was infused (3 mg/kg, over 10 min, i.v.) beginning either 10 min before (TY-12533-Pre, $n = 5$) or 10 min after (TY-12533-Post, $n = 5$) start of ischemia, and the group without treatment with TY-12533 was considered as control ($n = 5$).

[a] $P < 0.05$ vs. the value before ischemia.

racic muscle. The both electrodes were connected to a pH meter (CP-IPT, Inter Medical).

2.4. Experimental protocol

After hemodynamic stability, the left anterior descending coronary artery was occluded for 15 min followed by 2-h reperfusion. TY-12533 at 3 mg/kg was infused by means of a syringe infusion pump (1 ml/kg, over 10 min, i.v., Model-22, Harvard Apparatus, South Natick, MA, USA) started 10 min before (pre-occlusion treatment, $n = 5$) or 10 min after (post-occlusion treatment, $n = 5$) start of ischemia. The group without TY-12533 treatment was defined as control.

2.5. Statistics and exclusion criteria

Each value represents the mean ± S.E.M. Statistical analyses were performed using SPSS statistical package (SPSS Japan, Tokyo). Changes in the wall thickening and blood flow (after reperfusion) and in the pH (from 15 min before ischemia to 10 min after reperfusion) were compared between the control group and the TY-12533-treated groups by two-way analysis of variance for repeated measurements. Intra-group differences in hemodynamics were compared by Student's paired *t*-test and Bonferroni correction. Differences reaching $P < 0.05$ were considered to be statistically significant. Experiments were excluded from the final data analysis, if any of the followings occurred: (1) ventricular fibrillation (2) regional myocardial blood flow being more than 0.1 ml/min/g during ischemia (in order to avoid influence of blood supply through collaterals during ischemia).

2.6. Drugs

TY-12533 was synthesized by Drug Research Department, Toa Eiyo. The drug was dissolved in dimethyl sulfoxide (DMSO) and then diluted with 0.9% saline (final concentration of DMSO was 1%).

3. Results

Although pre-occlusion treatment with TY-12533 tended to elevate mean blood pressure (the value before TY-12533 infusion was 108 ± 4 mm Hg), mean blood pressure and heart rate were almost stable throughout experiments (Table 1). LVEDP increased during ischemia and returned to the pre-occlusion level after reperfusion in each group. LV dP/dt decreased during ischemia in the control group, and the decrease persisted after reperfusion. Reductions in LV dP/dt by ischemia were also observed in the TY-12533-treated groups, but the statistical significance was observed only 120 min after reperfusion in the pre-occlusion treatment group (Table 1).

In each group, ischemia reduced the wall thickening to about −30% (Fig. 1A). After reperfusion, the wall thickening in the control group gradually increased but remained depressed (the value after 30 min of reperfusion was about 40%). In contrast, either pre- or post-occlusion treatment with TY-12533 significantly enhanced the recovery of the wall thickening. The recovery was observed after 5 min of reperfusion, and values of the wall thickening after 30 min of reperfusion were about 90% in the TY-12533-treated groups. The blood flow was almost zero in all groups during ischemia, and hyperemic flow was observed after 5 min of reperfusion in all groups (Fig. 1B). Although levels of the hyperemia after 5 min of reperfusion were almost the same in all groups, the blood flow decreased below the pre-occlusion level (about 70%) after 30 min of reperfusion in the control group but not in the TY-12533-treated groups. The myocardial pH decreased during ischemia in all groups by the same extent (about 0.8 pH unit from pre-occlusion level, Fig. 1C). The recovery of pH in the TY-12533-treated groups tended to be faster

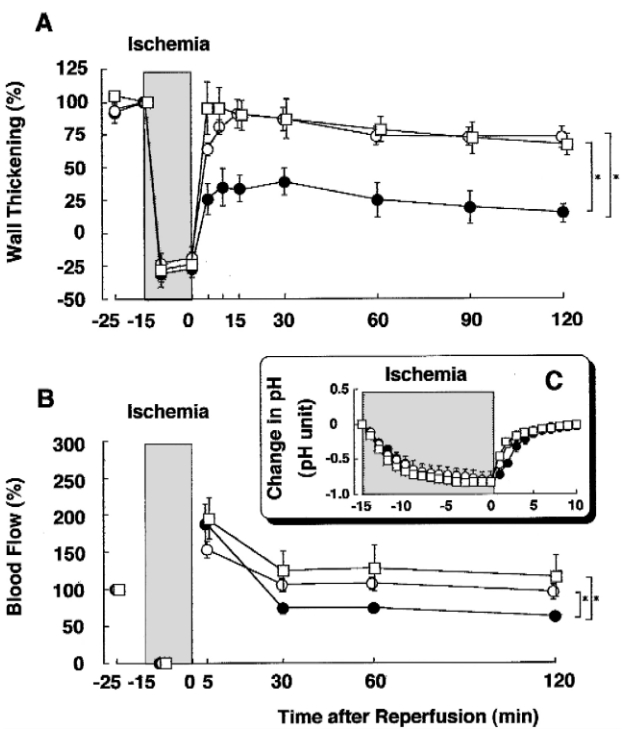

Fig. 1. The effect of TY-12533 on changes in myocardial wall thickening (A), blood flow (B) and pH (C) in the territory perfused by the left anterior descending coronary artery before and during ischemia (occlusion of the left anterior descending coronary artery) and after reperfusion. Values of the wall thickening and blood flow represent percent changes from the level before ischemia and the values of pH represent differences from the level before ischemia (mean ± S.E.M.). TY-12533 was infused (3 mg/kg, over 10 min, i.v.) beginning either 10 min before (○: pre-occlusion treatment, $n = 5$) or 10 min after (□: post-occlusion treatment, $n = 5$) start of ischemia, and the group without treatment with TY-12533 (●) was considered as control ($n = 5$). $^{*}P < 0.05$ vs. the control group after reperfusion.

than that in the control group but the differences were not statistically significant.

4. Discussion

Myocardial stunning is sometimes observed in clinical settings when coronary reflow is achieved by percutaneous transluminal angioplasty (Wijns et al., 1986) or by coronary bypass surgery (Breisblatt et al., 1990) after brief ischemia. In the present study, we examined the effect of a novel Na^+/H^+ exchange inhibitor TY-12533 on changes in myocardial wall thickening induced by 15-min occlusion of the left anterior descending coronary artery and reperfusion in dogs.

We simultaneously measured the myocardial wall thickening, blood flow and pH at the territory perfused by the left anterior descending coronary artery. The coronary artery occlusion reduced these values. Although the myocardial pH determined in this study by pH electrodes is the mixture of intracellular pH and extracellular (interstitial and blood) pH, the reduction of which could be an index for tissue acidosis induced by ischemia (Ichihara et al., 1979). After reperfusion, apparent coronary reflow was observed and the myocardial pH returned from the acidosis to normal level in each dog, but the substantial reduction in the wall thickening (about 50%) remained in non-treated dogs (control group). In our preliminary experiments, no histochemical changes were observed in myocardium subjected to 15-min ischemia followed by 2-h reperfusion (data not shown). Therefore, the residual reduction in the wall thickening after reperfusion in this study can be considered as the myocardial stunning as defined by Braunwald and Kloner (1982).

In animal experiments in vivo, a K_{ATP} channel opener (Auchampach et al., 1992), an L-type Ca^{2+} channel blocker (Ehring et al., 1992) and amiloride (Smart et al., 1995) prevented the myocardial stunning after brief ischemia. However, the results were obtained in pre-occlusion treatment (drug administration before ischemia), and the possibility cannot be ruled out that these drugs suppressed ischemic events, some of which could trigger the stunning, and thereby prevented the stunning after reperfusion. If so, these drugs might not be effective unless they were delivered to the cardiac tissue before onset of ischemia. Thus, it is still unknown whether these drugs prevent the stunning when they are used in post-occlusion treatment (drug administration during ischemia).

In the present study, neither pre- nor post-occlusion treatment with TY-12533 affected the reductions in the wall thickening and pH during ischemia, indicating that TY-12533 does not suppress the ischemic events such as myocardial dysfunction and acidosis. Nevertheless, TY-12533 in either pre- or post-occlusion treatment enhanced the recovery of the wall thickening and blood flow after reperfusion. These results demonstrate that TY-12533, even when administered during ischemia, is effective for preventing the myocardial stunning that occurs after brief ischemia. The effect of TY-12533 against the myocardial stunning may not result from the improved blood supply, because in the TY-12533-treated groups the wall thickening recovered after 5 min of reperfusion, at which the blood flow increased almost to the same extent in the control group and the TY-12533-treated groups. TY-12533 may therefore act on the myocardial cells to prevent the stunning. The inhibition of Na^+/H^+ exchange by TY-12533 may interfere with reverse-mode Na^+/Ca^{2+} exchange after reperfusion, and thereby suppress Ca^{2+} overload that is considered to be one of the major causal factors for the stunning (Bolli and Marbán, 1999). Our study is the first to show the possibility for clinical efficacy of the Na^+/H^+ exchange inhibitor on the myocardial stunning, which could also indicate a predominant role of Na^+/H^+ exchanger in pathogenesis of the stunning.

We have recently reported that the post-occlusion treatment with TY-12533 more effectively suppressed the ischemia/reperfusion-induced arrhythmias and myocardial infarction than the post-occlusion treatment with the authentic Na^+/H^+ inhibitor cariporide or the 9-Cl derivative of TY-12533 in rats (Aihara et al., 2000). We assume that TY-12533 is more sufficiently protonated to inhibit Na^+/H^+ exchanger during rapid recovery of tissue pH after reperfusion, because the pK_a value at guanidinium moiety of TY-12533 (6.93) is higher than the values of other two compounds (Aihara et al., 2000). This profile may also contribute to the efficacy of the post-occlusion treatment with TY-12533 on the stunning observed in this study.

In conclusion, this study demonstrated that either pre- or post-occlusion treatment with TY-12533 prevented the myocardial stunning induced by brief ischemia without affecting ischemic myocardial dysfunction or acidosis. Hence, TY-12533 is expected to be an effective drug in the new rational strategy for the treatment of ischemic heart disease.

References

Aihara, K., Hisa, H., Sato, T., Yoneyama, F., Sasamori, J., Yamaguchi, F., Yoneyama, S., Mizuno, Y., Takahashi, A., Nagai, A., Kimura, T., Kogi, K., Satoh, S., 2000. Cardioprotective effect TY-12533, a novel Na^+/H^+ exchange inhibitor, on ischemia/reperfusion injury. Eur. J. Pharmacol. 404, 221–229.

Auchampach, J.A., Maruyama, M., Cavero, I., Gross, G.J., 1992. Pharmacological evidence for a role of ATP-dependent potassium channels in myocardial stunning. Circulation 86, 311–319.

Bolli, R., Marbán, E., 1999. Molecular and cellular mechanisms of myocardial stunning. Physiol. Rev. 79, 609–634.

Braunwald, E., Kloner, R.A., 1982. The stunned myocardium: prolonged, postischemic ventricular dysfunction. Circulation 66, 1146–1149.

Breisblatt, W.M., Stein, K.L., Wolfe, C.J., Follansbee, W.P., Capozzi, J.,

Armitage, J.M., Hardsty, R.L., 1990. Acute myocardial dysfunction and recovery: a common occurrence after coronary bypass surgery. J. Am. Coll. Cardiol. 15, 1261–1269.

Ehring, T., Böhm, M., Heusch, G., 1992. The calcium antagonist nisoldipine improves the functional recovery of reperfused myocardium only when given before ischemia. J. Cardiovasc. Pharmacol. 20, 63–74.

Ichihara, K., Ichihara, M., Abiko, Y., 1979. Involvement of beta adrenergic receptors in decrease of myocardial pH during ischemia. J. Pharmacol. Exp. Ther. 209, 275–281.

Ogawa, T., Miura, T., Shimamoto, K., Iimura, O., 1996. Activation of adenosine receptors before ischemia enhances tolerance against myocardial stunning in the rabbit heart. J. Am. Coll. Cardiol. 27, 225–233.

Smart, S.C., LoCurto, A., el Schultz, J., Sagar, K.B., Warltier, D.C., 1995. Intracoronary amiloride prevents contractile dysfunction of postischemic "stunned" myocardium: role of hemodynamic alterations and inhibition of Na^+/H^+ exchange and L-type Ca^{2+} channels. J. Am. Coll. Cardiol. 26, 1365–1373.

Tani, M., Neely, J.R., 1989. Role of intracellular Na^+ in Ca^{2+} overload and depressed recovery of ventricular function of reperfused ischemic rat heart. Possible involvement of H^+–Na^+ and Na^+–Ca^{2+} exchange. Circ. Res. 65, 1045–1056.

Van Wylen, D.G.L., Willis, J., Sodhi, J., Weiss, R.J., Lasley, R.D., Mentzer Jr., R.M., 1990. Cardiac microdialysis to estimate interstitial adenosine and coronary blood flow. Am. J. Physiol. 258, H1642–H1649 (Heart Circ. Physiol. 27).

Wijns, W., Serruys, P.W., Slager, C.J., Grimm, M.J., Krayenbuehl, H.P., Hugenholtz, P.G., Hess, O.M., 1986. Effect of coronary occlusion during percutaneous transluminal angioplasty in humans on left ventricular chamber stiffness and regional diastolic pressure radius relations. J. Am. Coll. Cardiol. 7, 455–463.